THE

PUPAL STAGE OF CULEX,

AN

INAUGURAL DISSERTATION

FOR THE

DEGREE OF PH.D. IN THE UNIVERSITY OF LEIPZIG,

BY

C. HERBERT HURST,

Lecturer in the Victoria University,
Assistant Lecturer in Zoology in the Owens College,
Manchester.

WITH ONE PLATE.

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SHALLOW pools in most parts of Europe, and especially the smaller pools in woods, swarm in early spring with larvæ of Culex, hatched from eggs laid in floating masses by the impregnated females which have lived through the winter. These larvæ have been described by Swammerdam (1) and others, and most recently by Raschke (2).

After a few weeks the pupa escapes from the larval cuticle, and four days later the perfect insect flies free.

Though the pupal stage is the one of which I propose to give a fuller account than has yet appeared, it is necessary to the proper understanding of it that some account of the preceding and following states should also be given, and especially of the mode of life in each state.

The *larva* is an exceedingly active creature, swimming by a wriggling movement of the body, this being aided by a median fin-like series of setæ beneath the last segment. The head is provided with jaws and setæ, by means of which the solid food is collected and masticated. A pair of unjointed antennæ of considerable length arise from the sides of the head, and behind the base of each is a compound eye and behind this an ocellus.

The head is moveably attached by a neck to the broad, rounded thorax. The abdomen is long and slender, and composed,

apparently, of nine segments. The ninth segment bears four gill-plates surrounding the anus, and on its ventral surface, the median series of long setæ which serves as a propeller.

Respiration, according to Raschke, is performed in a threefold manner; by the gills just mentioned, by the rectum, and directly, the air being taken into the tracheæ by a conspicuous siphon projecting upwards from the eighth abdominal segment. The tracheæ of the abdomen serve not only as organs of respiration, but also by virtue of their great size as a float, keeping the larva when at rest at the surface of the water, with the hinder end uppermost and the end of the siphon touching the surface.

The alimentary canal is practically straight. The œsophagus is narrow. The stomach is wide, and extends from the anterior part of the thorax to the sixth abdominal segment. Its walls are very thick, and the epithelial cells very large, and in the thorax it has eight diverticula or pouches. From its hinder end the small intestine runs backwards to open into the wide rectum at the anterior end of the eighth abdominal segment, and this leads direct to the anus at the end of the body.

Five Malpighian cæca lie in the hinder segments of the abdomen, and open into the anterior end of the small intestine.

A pair of sac-like salivary glands lie at the sides of the stomach in the anterior part of the thorax, and their ducts, according to Raschke, unite, and have their opening "oben am Beginn des Œsophagus."

So far I have followed Raschke's account, except in ascribing a hydrostatic function to the "colossal" tracheal system, which is apparently much larger than would be necessary for respiratory purposes alone.

To Dr. Raschke's account I would add that, in addition to the head appendages, there appear during the larval period not less than eight other pairs of appendages beneath the larval cuticle. Of these six pairs are thoracic and two abdominal. The thoracic appendages are three pairs dorsal, the future pupal siphons, the wings and the halteres; and three pairs ventral, the future legs. The two abdominal pairs belong to the two last segments. Those of the eighth abdominal segment lie in the larval siphon, and form the fins of the pupa; the hindmost pair form the outer gonapophyses of the adult, which are accessory organs of copulation.

All these eight pairs alike arise as foldings of the epidermis ("hypodermis") outwards. All alike are completely hidden by the larval cuticle.

The antennæ, moreover, are much larger in an advanced larva than they appear to be. Their growth forwards being prevented by the unyielding cuticle, they grow backwards, and their basal portion is folded, and even "telescoped."

Towards the end of larval life the animal becomes sluggish; profound changes in its mouth-parts deprive it of the power of eating, and it floats with its siphon-stigma at the surface. Shortly the cuticle bursts in the thoracic region, along the mid-dorsal line; the pupal "horns" or siphons are protruded, the abdominal tracheæ appear to collapse, and the animal floats with the anterior end upwards, the new siphons coming to the surface. The old larval siphon, or rather its soft parts, are withdrawn from the cuticle and *invaginated into the eighth segment of the abdomen;* the intima of the abdominal and thoracic tracheal trunks breaks up into pieces, which in the abdomen correspond to body-segments. The body of the escaping pupa is gradually withdrawn from the larval cuticle, and the eighteen fragments of the old tracheal intima are drawn out of the body by nine pairs of stigmata, and cast off with the exuviæ. These nine pairs of stigmata are situated, one in the hinder part of the thorax, one in each of the first seven segments of the abdomen, and the ninth pair are united to form a single aperture, the old respiratory opening at the end of the larval siphon.

With the larval exuviæ are also cast off the cuticular portions of the jaws and antennæ, and all the hairs and spines with which the larval cuticle was beset.

The *pupa* which thus escapes differs from the larva very widely. It is a little under 1 cm. in length when fully extended. It consists of a bulky, laterally compressed mass made up of head and thorax with their appendages, and of a slender flexible abdomen, which when at rest is curved under the thorax. In a specimen measuring 9 mm., which is nearly the maximum size, the thorax measures 2·5 mm. and the abdomen 6·5 mm., but the thorax appears to be much longer on account of the wings which extend downwards and backwards from its sides.

The head lies below the thorax, and so adds nothing to the length

of the animal. It is broad from side to side, short from back to front, while ventrally it is drawn out into a long process which extends backwards under the thorax as far as the anterior part of the abdomen, where it curves upwards. This process is made up of what are usually spoken of as the "mouth-parts," including labrum, epipharynx, one pair of mandibles, two pairs of maxillæ, and the hypopharynx. The second pair of maxillæ are fused together to form the labium.

In describing an animal which is coiled up so that head and tail almost meet, the terms "dorsal," "ventral," "anterior," and "posterior" are liable to be misleading. To avoid this as far as possible I shall apply the terms to those parts to which they would be respectively applicable in the fully-developed insect in the act of sucking blood, *i.e.*, I shall regard the general direction of the mouth-parts as downward, their distal ends as ventral, and I shall speak of the labrum as being in front of the mouth.

From the sides of the epicranial region the antennæ run outwards to the sides of the thorax, and then downwards, one beneath the anterior margin of each wing. The head and all its appendages are immoveable during pupal life.

The thorax is rounded, but somewhat compressed from side to side. From the sides of its summit arise the respiratory siphons, a pair of conspicuous organs whose position has led to the name "horns" being applied to them. The wings are nearly flat oblong plates, arising behind the bases of the siphons and extending downwards and backwards. Immediately behind them are the halteres, a pair of triangular plates enclosing the halteres of the future gnat. I have endeavoured to show the forms of these parts in Fig. 1.

The legs are almost completely hidden by the wings, but the femur, tibia, and first joint of the tarsus of the first leg, and the tibia and first joint of the tarsus of the second are visible (Figs. 1 and 2).

The abdomen is flattened dorso-ventrally, and when at rest is curved under the thorax. It is jointed and flexible, and forms with the pair of large flat fins borne by its eighth segment the only locomotor organ of the pupa, the wings and legs lying immoveable and even adhering to one another, though they are easily separated in specimens which have been kept in alcohol.

The pupa does not eat. It breathes air through the apertures at the ends of its siphons. It floats, thorax uppermost, by virtue of a large air cavity lying under the hinder part of the thorax and the anterior part of the abdomen. This cavity is bounded in front by the legs, at the sides by the wings, and below by the mouth-parts. It extends up at each side of the first segment of the abdomen, where it is covered by the halteres, and into this part of the cavity at each side opens a large stigma, held open by the fairly well-developed cuticular lining ("intima"), and guarded near its entrance by numerous spines. These two stigmata belong to the first abdominal segment, and put the air-cavity just described into direct communication with the tracheal system. As already mentioned, I regard this cavity and these stigmata as being mainly, if not exclusively, hydrostatic in function, serving not only to make the pupa float when at rest, but to make it float in a definite position, with the thorax uppermost and the apertures of the siphons at the surface of the water.

The pupa is sensitive to light, and immediately darts backwards when a shadow falls upon it suddenly. The movements, however, though very rapid, are devoid of anything like steering. The larva had to steer in its search for food, but the pupa has simply to get out of the way of danger, and the direction of its flight is of little importance, though, since the movement is always backward with reference to the pupa, it is chiefly downward with reference to the outer world.

A sudden very loud noise, or a very gentle tap upon the vessel containing the pupæ, causes those at the surface to dart downwards, but as slight sounds of various kinds produce no effect upon them, I conclude that the tremor of the surface of the water, and not the sound itself, was recognised by them. The setæ on the first segment of the abdomen are probably the organs by which this movement is felt.

As to the anatomy of the pupa, it is only necessary now to state that at the beginning of pupal life the internal arrangements are those of the larva; at the end of that period they are those of the imago.

At the beginning of the fifth day of pupal life, the cuticle splits along the mid-dorsal line of the thorax; the thorax of the imago

protrudes, and the head, then the abdomen, and lastly the wings, legs, and proboscis are drawn out of the pupal cuticle, which is left floating in the water while the imago flies away.

With the cuticle are cast off nine pairs of fragments of tracheal intima, two pairs being drawn out through the thoracic stigmata, the others through the stigmata of the first seven segments of the abdomen.

These fragments differ as follows. The first pair are well developed, and have the spiral thickenings very well marked. They are continuous with the lining of the respiratory siphons, and formed during pupal life the connection between these organs and the tracheal system generally.

The first abdominal pair are not so well developed, but the spiral thickening is recognisable in them, and the terminal portion of each is better developed than the rest, and is beset internally with numerous small spines. It was through these that the tracheal system communicated during pupal life with the air-cavity beneath the thorax. The remaining fragments, *i.e.*, the hinder thoracic pair and all the abdominal pairs except the first, are very thin and delicate, and were functionless during pupal life.

The cuticular lining of the anterior and posterior portions of the alimentary canal and the cuticle of the invaginated larval siphon are also shed, together with all setæ and the whole of the pupal siphons and fins. These three last alone involve the loss of portions of tissues other than cuticle.

The imago, with its long, slender body, wings, legs, and proboscis, hardly needs to be described. Like pupa and larva it breathes air, but now by more numerous stigmata, and unlike them it flies in the air. The larva fed upon solids ; the pupa did not eat at all. The imago feeds upon fluids, and the female, at least, upon the hot blood of man and other mammals. The male is short-lived, and his food is said to consist of the sweet juices of flowers. To find the female and to impregnate her are the real objects of his short life. His antennæ are provided with long hairs, which A. M. Mayer (3) has shown to be sensitive to a particular sound when the head is turned towards the source from which it proceeds, and he has further shown that sound to correspond to the note emitted by the vocal organs which Landois (4) has described on the sides of the thorax

of the female, beneath the halteres. The male, therefore, would appear to be endowed with a special and very largely developed pair of organs for detecting the whereabouts of the female.

The female imago, after impregnation, has to find a suitable place to lay her eggs, *i.e.*, the surface of a stagnant pool; and having found it, has to lay the eggs at a suitable season, and this in the case of females escaping from the pupa late in the summer involves the necessity of living through the winter. Hence the mouth appendages are specially adapted for piercing the skin and extracting the blood of mammals, and this is stored in her capacious stomach. The structure of the mouth-parts in the two sexes has been described by Dimmock (5); but as he appears uncertain as to the injection of "saliva" into the wound, I shall add that a special apparatus developes during pupal life by which the saliva is discharged near the tip of the hypopharynx ("lingua").

Having now given a brief outline of the life-history of the gnat, I will proceed to describe the pupa and the changes which it undergoes in more detail. As, however, I have directed my attention almost exclusively to the more important organs of the body, and not to hairs and the like, I shall not make any distinction of species. What I have to record is probably applicable to all species alike.

DESCRIPTION OF THE PUPA OF CULEX.

The External Characters.

The *head* is broad from side to side; the epicranium has a well-marked median groove; the clypeus, broad above, is gradually narrowed below, and continued without any distinct line of demarcation into the labrum. At the sides are a pair of compound *eyes*, to be regarded rather as the rudiments of the eyes of the future gnat than as the visual organs of the pupa itself. Their form and size in the earliest stage are shown in Fig. 1. During pupal life they increase in size till they almost encircle the head. Corneal facets are never formed in the pupal cuticle, but beneath it the convex facets of the imaginal cornea are formed during pupal life.

Behind the compound eye, on each side of the head, is an *ocellus* with fully-developed lens, etc. In the youngest pupæ it is separated by a small interval from the compound eye (see Fig. 1); but the growth of the latter obliterates this interval, and the ocellus is in the older pupæ not readily distinguishable except in sections. The statement, found in systematic works, that the Tipulariæ are devoid of ocelli is, however, not strictly true; in Culex, at least, they are well developed, though, as they abut upon the compound eye, they are in the imago so inconspicuous that they may easily be overlooked.

In the *mouth-parts*, the labrum, epipharynx, mandible, maxillæ with their palps, labium and hypo- and epipharynx are present, though the two last can only be seen on dissection.

Of their mode of origin in the larva I as yet know nothing. At the time of escape of the pupa from the larval cuticle they are of the full size, which is considerably greater than in the adult. The form of most of these parts is shown in Figs. 1 and 2. That I may not have to refer to these parts again, I will at once say that the chief changes which occur in them during pupal life are:—(1) The development of a cuticle within the pupal cuticle, and this, in the case of the labium (fused second maxillæ), is covered with scales closely resembling those found in Lepidoptera; (2) a considerable shrinking; (3) in the male only, atrophy of the mandibles, which in a young pupa are as large as in the female, but in the adult are not recognisable.

The *antennæ*, which were folded and telescoped at their bases in the larva, are in the pupa laid upon the sides of the thorax, as seen in Fig. 2. Their hinder (distal) extremities are hidden by the wings. The swollen basal joint of the antenna of the imago is hardly recognisable on the surface, although it is already a conspicuous object in sections of the youngest pupæ, and even in the larval state. I shall describe it with the other sense organs. The shaft of the antenna is segmented, but the external segmentation loses its correspondence with the segmentation of the developing antenna within it early in pupal life.

The *thorax* is large and rounded, but somewhat compressed from side to side. Mid-dorsally the cuticle of the prothorax is marked by fine transverse corrugations, and this is the part which ruptures to

allow the imago to escape. A pair of branched setæ arise from the dorsal region of the hinder part of the thorax.

The *respiratory siphons* (AT, Fig. 1) are nearly cylindrical, narrowed at their bases and curved forwards to be attached by flexible membranes to slight prominences on the sides of the prothorax. Above they are obliquely truncate and open, and the margin is slightly notched on the inner side. The outer surface is marked so as to resemble imbricated scales, each with a minute spine at its apex. The cavity of the siphon communicates directly with that of a tracheal trunk at its base. Palmén (6) says that after a "close investigation" he has found that there is no opening. The tone of assurance in which he contradicts all previous observers led me to put the question to the test. I removed the side wall of the thorax, with some of the underlying muscles and tracheæ, from a specimen preserved in alcohol. I drew out the alcohol from the cavity of the siphon by means of blotting-paper, and then touched the tip with a minute drop of glycerin. I watched the effect under the microscope, and saw the glycerin force its way into the siphon, driving the air before it into the tracheæ. Palmén, moreover, says the organs are gills! Each is a thick chitinous tube, the cavity guarded by numerous hooked spines, the walls consisting of hardly anything but the chitinous cuticle, the epidermis ("hypodermis") between its two layers being barely recognisable on account of its thinness. The "tracheal gills" on which Palmén lays much stress have absolutely no existence.

The *wings* of the pupa, that is the organs within which the wings of the imago are developing, are a pair of oblong plates about 2½ mm. in length. They are closely applied to the sides of the hinder part of the thorax, and directed downwards and backwards. They are immoveable.

The *halteres* are a pair of elongated triangular plates lying along the dorsal and hinder border of the wings.

All these three pairs of dorsal appendages arise within the larva in the same way, and their bases or points of attachment all lie in the same horizontal plane. Each is at first (in the larva) a fold of the epidermis; each acquires a cuticular covering (like all other parts of the body), and the first pair become rolled up to form tubes, the respiratory siphons, while the other two remain flat plates.

The three pairs of ventral appendages of the thorax, or *legs*, are long cylindrical bodies folded upon themselves, and lying beneath the thorax and between the wings. The same segmentation into femur, tibia, etc., is recognisable as in the adult gnat, but the segments are more nearly equal in the pupa, and the joints of the developing and shrinking legs of the future imago soon lose their correspondence with those of the pupal cuticle enclosing them. They arise in the larva, like other appendages, as folds of epidermis enclosing mesoblastic tissues.

The *abdomen* is dorso-ventrally compressed and exceedingly flexible dorso-ventrally, though not from side to side. It is the only part of the pupa in which the segmentation of the body is readily recognisable, and as I shall very frequently have to refer to the various segments by number, *I shall use the terms "first segment," etc., to signify "first segment of the abdomen," etc.*

Nine segments are readily recognised in the abdomen, and the last one, though it is probably composed of no less than three condensed and highly modified segments, I shall call simply "ninth segment."

Each abdominal segment has a chitinous tergum and sternum, and setæ are distributed sparingly over them, being almost confined to the hinder parts of the terga. The terga and sterna of successive segments are united by soft arthrodial membranes.

Of the *setæ*, only one pair need special mention. These are placed on the hinder part of the first segment, the base of each being a triangular plate attached by one angle to a soft membrane, and the distal side of the plate is divided into a number of bars which, by repeated division or branching, give rise to about one hundred setæ all lying in one plane parallel to the median plane of the body. Each seta bears a few fine hairs. When at rest, the pupa floats with the tips of these setæ, and the tips of the respiratory siphons, at the surface of the water, and these setæ probably assist in maintaining the equilibrium of the animal in this position, as well as serving as sensory organs by means of which any disturbance of the surface is felt.

The eighth segment bears a pair of large *fins*, thin oval plates about 1·2 mm. in length, attached by the narrow end beneath the tergum behind. Each is stiffened by a midrib which projects beyond the hinder border of the fin as a spine. (Fig. 2.)

Beneath the fins and behind the eighth segment is the "*ninth segment*" with its appendages. Though this region is probably made up of more than one segment, its composite nature is not easy to recognise, as the plates supposed in other insects to represent the terga and sterna of tenth and eleventh segments [see, for instance, Huxley (7) and Miall and Denny (8)] are not developed in the young pupa, nor, indeed, is there in any stage any such development of the pupal cuticle, though plates developed within as parts of the imaginal cuticle may perhaps represent some of these parts.

The appendages of the "ninth segment" of the pupa are a pair of blunt processes arising below and in front of the anus, and directed backwards below the fins. They are much larger in the male than in the female. A pair of appendages are already recognisable in this region in sections of the larva, and I think even two pairs, but this portion of the larva is particularly difficult to cut, and I am not yet certain as to the hinder of the two pairs. Of the existence of one pair I have no doubt.

The Digestive System.

The *alimentary canal* of the pupa runs almost direct from end to end of the body, the only convolution occurring in the region of the intestine.

In the youngest pupa the condition is practically that of the larva. (*See* Raschke, *op. cit.*) The narrow œsophagus projects slightly into the stomach. The stomach extends from the anterior part of the thorax to the end of the fifth segment (abdominal): it is very wide, and the eight diverticula found in the thoracic region of the larva are still present. The walls are very thick, and the cells of its epithelium large.

The stomach opens behind into the intestine, which is slightly coiled and opens into the wide rectum, which ends at the hinder end of the abdomen. The epithelium of the rectum consists of very large cells, and is thrown into longitudinal folds.

The *salivary glands* are unbranched sac-like glands in the anterior part of the thorax at the sides of the alimentary canal. Their ducts unite beneath the sub-œsophageal ganglia, and from this point the single median duct runs forwards to open in the floor of the mouth.

The five *Malpighian cæca* open into the anterior end of the intestine. They are nearly cylindrical bodies of an intense white colour; their closed ends lie in the seventh or eighth segment, and measure about 0·13 mm. in diameter. They run forwards, almost straight, to near the anterior end of the fourth segment, and then backwards to their point of opening into the intestine immediately behind the constriction dividing the latter from the stomach. The diameter of each near its opening is about 0·03 mm. Each cæcum is made up of two rows of cells alternating more or less regularly on the two sides, the narrow lumen taking a zigzag course between them. The individual cells are very large, the long diameter of each being the diameter of the organ itself. The nucleus is large and transparent, but the rest of the cell contains a large quantity of a granular deposit which gives the organs their intense white colour. During pupal life I have noted no important changes in these organs.

Such is the structure of the alimentary canal and its appendages at the commencement of pupal life—a structure adapted to the life of the larva, but not to that of the imago, and the changes which it undergoes during the pupal period are so great that at the end of that period no part of the whole canal corresponds in structure to the above description.

The most striking change is the reduction in thickness of the epithelium which occurs throughout, but which is perhaps best shown in the stomach. Four stages of the change are shown in Figs, 3, 4, 5, and 6, which are drawn from the epithelium of the hinder part of the stomach. The beginning of the change has already occurred before the pupa leaves the larval exuviæ, but the first stage here shown (Fig. 3) is from a young pupa. At the base of each of the large epithelial cells may be seen one or two nuclei; a little later the protoplasm of the cell divides into a small portion around the new nuclei, and a much larger portion, which rapidly undergoes degeneration and, separating from the basal layer (the new epithelium), is apparently digested. The outer surface of the stomach is covered by an exceedingly thin layer in which I could not make out any structure, but which is presumably muscular, and is at first folded longitudinally (Figs. 4 and 5), but afterwards becomes even, the new cells at the same time becoming flattened (Fig. 6).

A similar change occurs in the intestine. The epithelium divides into a thin outer and a thick inner layer. The latter becomes loosened, breaks up, and appears to be digested.

In the rectum more complex changes occur, though here also the superficial portion of the epithelium is thrown off, but it breaks up later and more slowly than elsewhere; in fact, the disintegration and digestion appears to commence in the anterior part of the stomach, and progress gradually backwards. Before the epithelium shows any sign of disintegration in this region, the rectum becomes differentiated into two parts: an anterior very wide part, the "rectal pouch," and a narrower hinder portion, to which alone I shall apply the term "rectum" from this stage onwards. The wall of the rectal pouch rises up into four very large and prominent papillæ, the "rectal glands": one ventral at the anterior end of the pouch, just below the opening of the intestine into it; one dorsal and posterior, and two lateral, intermediate in position between the other two. Nerves and tracheæ push their way into the axis of each papilla. The epithelium of the papillæ undergoes division into two layers as elsewhere, but the distal layer, which is ultimately shed, is very thin, and the basal or permanent epithelium consists of very large columnar cells, while the opposite is the case everywhere else, and especially in the rectum, where the permanent epithelium is so thin that I had difficulty in detecting it, and Chun (9) states that it is absent in *Musca* and other insects.

Besides this shedding of epithelium, changes of form occur in various parts of the alimentary canal.

The anterior part of the œsophagus expands, especially in the female, and acquires a thick chitinous lining. In cross section it becomes triangular, and the sides and roof, all of which are convex inwards, are supplied with muscles arising from the walls of the head, which by their contraction increase the size of this cavity, and serve to produce the sucking action by which the imago draws the blood of its victims up through its proboscis. This apparatus is not well-developed in the male.

The posterior part of the *œsophagus* gives off ventrally a large diverticulum ("crop"), which runs backwards under the stomach as far as the hinder end of the thorax, its walls developing numerous small sacculations along its two sides. Sometimes a forwardly-

directed diverticulum of this crop is found arising from its ventral wall. The crop appears at a later stage than that shown in Fig. 7.

The cavity of the *stomach* becomes wider, while the part behind it becomes narrower, with the exception of the rectal pouch.

The *salivary glands*, which at the beginning of pupal life were a pair of hollow unbranched club-shaped organs lying at the sides of the alimentary canal in the anterior part of the thorax, become during pupal life divided into about four branches, and the cavity almost disappears, and acquires a pretty thick chitinous lining. The *ducts* run downwards to the neck, which they traverse at the sides of the nerve cords. Just below the hinder border of the sub-œsophageal ganglia they unite to form a median duct, which runs forwards to open into a pit at the base of the hypopharynx. This pit becomes deeper during pupal life, and acquires a very thick chitinous lining. From it a deep groove, also *very* strongly chitinised, runs downwards along the middle line of the anterior surface of the hypopharynx to its extremity. This is true of both male and female; but the hypopharynx of the male is inseparable from the labium.

The Circulatory System.

The *heart* lies in the abdomen in a median space between the extensor muscles and close beneath the dorsal wall of the body. It arises at the anterior end of the eighth segment, and ends suddenly at the anterior end of the first segment, giving off the aorta from the ventral border of its anterior end. From its sides "alæ cordis" run outwards beneath the extensor muscles and between the main tracheal trunks and the stomach, to be attached to the "peritoneal" covering of the tracheal trunks, or to the outer layers of the wall of the stomach. Each "ala" consists of a dorsal and a ventral lamina, the two running together some distance from the heart. The space between them has been called "pericardium": it contains the "pericardial cells," and communicates freely with the body cavity by the spaces between the alæ. The ventral lamina of each is continuous with the corresponding lamina of the other side of the body, and all the ventral laminæ together thus form an imperfect "pericardial septum" (Graber). The dorsal laminæ are attached to the sides of the heart near the dorsal surface, their fibres taking a

longitudinal direction on the heart, and forming its outermost layer. The heart is further bound by fibrous strands to the dorsal body wall.

Graber (10) appears to believe that the septum is invariably attached to the outer wall of the abdomen, dividing the cavity of the abdomen into two cavities, a small dorsal "pericardium" containing only the heart and pericardial cells, and a large ventral cavity containing all the other organs of the abdomen. His figure of Acridium is reproduced in the most popular text-book (Claus), and his view that this arrangement is universal, and that the "septum" serves as a pump driving blood from the large abdominal cavity to the pericardium, is reproduced in other text-books, in such form as to lead to the belief that the arrangement is the same in all insects. Whatever may be the case in other insects, this view is certainly not applicable to Culex. Here the "septum" does not extend to the body wall, and if a "pericardium," in Graber's sense of the term, exist at all, the extensor muscles and the main tracheal trunks lie in it, and the septum cannot, judging from its anatomical relations, have the function ascribed to it.

The heart itself is a more or less cylindrical tube, about 0·06 mm. in diameter. Its hinder end at the anterior limit of the eighth segment is open, but I am unable to give an account of any valvular apparatus which may be present here. There is no sharp division into chambers either by constrictions or by valves. In the first segment a pair of valved ostia opens *backwards;* in segments III to VII paired ostia are present, their margins being turned in and directed *forwards* to form the valves. I have not detected any aperture or valve in the second segment. The ostia are small paired slits in the sides of the heart, and between the two laminæ of the alæ, putting the cavity of the heart in communication with the "pericardial" cavity. The infolded margins of the slits serve as valves in two ways; firstly, they prevent the blood from flowing out through the ostia; and, secondly, they prevent the blood within the heart from flowing backwards during systole.

Of the histology of the heart I would speak with the greatest caution. Graber (*op. cit.*) has made the subject his own, and has applied very special methods to the investigation. My object has been rather to record the anatomical structure and the development

of the pupa, and I simply note histological results incidentally, referring those who wish to learn the histological structure of insect hearts to the classical work just mentioned.

Without the application of special methods, I have recognised three layers in the wall of the heart.

The inmost layer, or *endocardium*, is an exceedingly thin layer of flat cells. Their nuclei are conspicuous objects, occurring with striking regularity in pairs, four pairs to each segment of the abdomen, and a similar but smaller nucleus is to be seen in each flap of each valve, from which I conclude that this endocardium extends also to the valves. Whether the other layers also extend into the valves or not, I cannot say with certainty.

The *middle layer* consists of encircling fibres, slightly oblique in direction, and probably muscular.

The *outer layer* is also fibrous, its fibres being on the whole longitudinal in direction, but they curve outwards to be continuous with the fibres of the dorsal laminæ of the alæ.

Between the laminæ of the alæ cordis, that is in the pericardial cavity, are large ovoid masses of brown cells, the "*pericardial cells.*" Of these masses there are two pairs near the anterior, and two near the posterior end of each segment of the abdomen; but the number increases towards the end of the pupal stage, and still further in the imago, by the division of some of them into two or more masses. The protoplasm of these cells is extraordinarily spongy, and contains numerous granules, which stain deeply with borax carmine. The nuclei vary in number from three or four to ten in each mass, though the boundaries of so many cells cannot be made out. The cells appear to be undergoing division very slowly. The excretory function of these cells has recently been shown by Kowalevsky (12).

The *aorta* runs from the ventral border of the anterior end of the heart forwards above the stomach and œsophagus to the head, where it ends, the end being open. In transverse sections of the thorax, the aorta is seen as a laterally compressed tube. I have not seen any branches given off from it.

The Respiratory System.

Culex, as already mentioned, breathes air in all three states—larva, pupa, and imago—and also breathes it directly, but the air is

taken in at different apertures in the three states. The larva, according to Raschke (*op. cit.*), breathes also by gills and by the rectum.

I have already described the respiratory siphons of the pupa, and given evidence to show that they really do lead directly into the tracheæ, in spite of Palmén's contention to the contrary.

From the base of each siphon, tracheæ run to various parts of the body and head. Amongst these may be mentioned specially one transverse trunk running across the thorax between the alimentary canal and the nerve-chain, and putting the two siphons in direct communication with each other; and a pair of longitudinal trunks running backwards to the hinder end of the body, and giving off branches to the various organs, and also a trunk to each of the stigmata. As already mentioned, these stigmata are present in the hinder region of the thorax, and in each of the first seven segments of the abdomen; but the stigmata, except the first abdominal pair, are closed, and the pupal intima of the tracheæ connecting them with the main trunks is thin and collapsed. The widely open stigmata of the first segment, with their spines and their probable function, I have already commented upon; but while insisting on the importance of the hydrostatic function of the tracheal system in both larva and pupa, I would again say that I do not consider this a sufficient ground for the view that the hydrostatic function is the primitive one. In Culex larva and pupa, it is important only inasmuch as it subserves respiration by bringing the animal to the surface and maintaining it there in the only position in which air can be breathed directly.

The cuticular lining ("intima") of the chief trunks and their branches is well developed even at the commencement of pupal life, and has the usual spiral thickening. The trunks connecting the stigmata with the main trunks are the only ones that undergo any marked change during the pupal condition. These widen around their separated and collapsed intima, and a new and strongly thickened intima is formed. In the main trunks no new intima is formed, and when the imago escapes from the pupal cuticle no portion of the intima is shed from any part of the system which has been functional during pupal life, excepting the portions connecting the siphons and the first abdominal stigmata with the main trunks. These fragments are, in the case of the siphons, well developed, and

have a fully-developed spiral thickening. The portions connected with the first abdominal stigmata, though better developed than the portions connected with the other stigmata of the abdomen, have the spiral thickening only slightly developed. The terminal portion is beset with very numerous small spines.

The fate of the invaginated portion of the larval siphon is interesting. The whole of the tissues composing it break up and undergo complete absorption, so that no trace of it is discoverable in the advanced pupa.

Before dismissing the respiratory system, I will again state that the pupa breathes air only, and breathes it through the open stigmatic horns or siphons alone. The *tracheal gills* of Palmén have no existence.

The Muscular System.

Concerning this I have nothing new to communicate. The muscles of the pupa are those of the imago. All the chief ones are present in the young pupa, but they increase greatly in size, and this is especially true of the thoracic muscles.

The Nervous System.

The *nervous system* is particularly interesting. Within the short space of four days, certain ganglia increase enormously in size by the addition of cells apparently derived directly from the epidermis; and other ganglia, already well developed and functional, shift bodily from their original positions, and in some cases fuse with ganglia originally remote from them.

Raschke (*op. cit.*) says that in the larva each of the first eight segments (of the abdomen) has a pair of ganglia, and this statement is certainly true of all the larvæ I have examined, and yet a pupa which I killed when only half escaped from the larval cuticle had already four pairs in the thorax, and none in the first segment of the abdomen. During pupal life these four ganglionic masses fuse into one compact mass, though its composite nature is always recognisable in sections.

The ganglia of the eighth segment (of the abdomen) at the beginning of pupal life occupy their typical position in the anterior part of the segment, and are connected with the ganglia of the seventh segment by connectives (or "commissures") nearly equal in

length to the seventh segment. During the first two days of pupal life these connectives vanish completely, and the ganglia migrate to the anterior part of the seventh segment to fuse with the ganglia of that segment. As with other composite ganglionic masses, the composite nature of the ganglionic mass so formed is easily recognised in sections, especially horizontal sections, even in the imago. In the female the process goes a stage further. A pupa almost ready to burst and give exit to the imago has still the arrangement just described, but the imago, killed immediately after its escape, is found to have no ganglia in the seventh or eighth segment, but in the sixth segment are two masses; the first, the pair properly belonging to the segment, lying at its anterior end; the other, the double ganglionic mass formed by the fusion of the seventh and eighth ganglia, lying at the hinder end of the segment.

In the *male* imago, however, the arrangement is the same as in the advanced pupa.

A detailed description of the ganglia of the head and the changes they undergo during pupal life would take me too far. The most striking change is the very great increase in size which these ganglia undergo, and the most interesting point is the way in which this increase is brought about. The epidermal ("hypodermal") cells, especially those near the borders of the eyes, proliferate freely, and the cells budded off from their inner surfaces migrate inwards and form the new cells of the ganglia. By this process the ganglia, which at the beginning of pupal life were comparatively inconspicuous, grow till they almost fill the head, and there are places in the advanced pupa where the ganglia and the epidermis appear to be continuous.

The *sense-organs* of the pupa itself are not of special interest, that is, the organs which serve during pupal life as sense-organs. The *setæ* have already been mentioned. The *ocelli* are those of the larva, but they persist to the adult condition, the chief change which they undergo being the development of an exceedingly dense pigment. I have already referred to the common but erroneous statement that the imago is devoid of ocelli.

The *compound eyes* belong properly to the imago, not to the pupa, though they are probably sensitive to light in the pupal condition. At first they are small (see Fig. 1) and devoid of corneal facets, but

they grow till they occupy the greater part of the surface of the head.

So much has of late been written upon the eyes of insects, that one should hesitate to add to that literature without having made very special study of the organs in question. Still, one of the most remarkable papers of the day (Patten, **11**) has attracted so much attention, and is so strongly opposed to the views of previous observers, that the little I have already observed may be of interest.

Each eye is made up of a very large number of "elements." Growth of the eye consists in the addition of new "elements" at its edge. Each new element is formed directly from the previously unmodified epidermis at the margin of the eye, and each arises independently of the rest of the eye, as a separate invagination of the epidermis. The cells, four in number, around the margin of each invagination, persist as the "nuclei of Semper," "corneal hypodermis," "corneal epidermis," "cellules cristallines," "cellules de Semper," "refractive globules" or "spherules." The invaginated portion gives rise to all the other parts lying outside the limiting membrane, with the possible exception of the pigment cells. The elements are at first devoid of pigment.

The details of the development I have not yet worked out, and I think it best to reserve further description for a future paper.

Other sense-organs developed during pupal life are *antennæ*. Antennæ are, it is true, present already in the larva, but they have no resemblance to those of the imago, and they are functionless during pupal life.

The epidermis round the base of each antenna of the larva grows rapidly, and as it is prevented, by the rigid and unyielding cuticle of the shaft of the antenna, from growing forwards, it grows backwards, and becomes "telescoped" and much folded, and sections through the larva show that the differentiation of the epidermis of the different parts has already begun.

When the pupa escapes from the larval cuticle, much of the folding is undone, but a portion of the telescoping persists at the base of the organ, and this part gives rise to the large hemispherical basal joint of the antenna of the imago.

This remarkable organ was described in the imago thirty-five years ago by Johnston (**13**), but very imperfectly. Externally it is

not conspicuous in the pupa, though it is just recognisable. During pupal life its parts undergo considerable change, and these will be best understood if I describe the adult structure first.

In the imago the antennæ differ markedly in the two sexes. In the female the shaft is longer than in the male, and the hairs with which it is beset are less numerous and very much smaller. In both sexes the basal joint is enlarged, and forms a nearly hemispherical cup, with small cavity and very thick walls, covered and lined with chitin. The shaft of the antenna arises from the centre of the cup, and the chitinous floor of the cup is strengthened by a series of radial thickenings. In the female the edge of the cup is turned in, so that the aperture of the cup is narrower than the cavity immediately below. The structure in the male is really an exaggeration of this; the edge is folded in so completely that it unites with the floor, and the walls of the cavity of the cup of the female thus come to be represented by a concave double disc, the two laminæ of which are closely united, and the space between them, the equivalent of the cavity of the cup in the female, is here obsolete. The attachment of the shaft to the floor of the cup appears to be rigid, and the organ would appear to be adapted for the perception of sound-waves coming in the direction of the axis of the shaft alone.

A section taken along the axis of the organ shows the following structures: A layer of flattened epidermal cells, next to the cuticle of the outer wall; then a layer of cells I shall call "ganglionic," thickest at the base of the cup, and continuous with the antennary lobe of the "brain." Between this layer and the inner wall of the cup is a double (perhaps treble) layer of long narrow rod-like cells, at right angles to the surface, that is, radiating from the centre of the cup.

These structures form a thick ring round the cup, perforated at the base by the nerve supplying the shaft of the antenna.

The basal joint is supplied by an enormously large nerve arising from the ventral portion of the supraœsophageal ganglion at the side of the œsophagus. This nerve is broader than the abdominal double nerve cord, and is independent of the nerve supplying the shaft of the antenna, which lies ventral to it. The nerve, after entering the organ, divides, one layer penetrating the "ganglionic" layer; another runs between the ganglionic layer and the layer of rods, and a third

on the inner surface of this layer, supplying certain small rounded cells lying between this layer and the base of the shaft.

All the cellular layers of this organ are epidermal in origin, but the layer which I have called ganglionic stands, during the later part of pupal life, in direct continuity with the superficial layer of cells of the "brain," and this layer in turn is continuous with the deep layer of the epidermis of the head immediately behind the base of the antenna. Whether the continuity of the ganglionic layer of the organ with the brain is due to identity of origin, both being budded off from overlying epidermal cells, or to migration of cells from the brain into this organ, is difficult to determine, but the cells of the cerebral lobes ("hemispheres") are larger than those of the cup-like organ, while the latter resemble the cells of the inner optic lobes very closely in size and in mode of staining.

This organ is already a conspicuous object in sections of the larva, more conspicuous indeed than the "brain," but the differentiation of the layers is only completed late in pupal life.

The Reproductive System.

I. The *male* generative organs of the adult consist of testes, vasa deferentia, "prostatic glands," copulatory organ with a common pouch at its base, and two pairs of gonapophyses. Of these last the outer ones are a large pair of forceps for holding the female. Both pairs are probably segmental appendages, and I have already spoken of their origin in the larva.

The testes are a pair of cylindrical bodies already present in the larva at the sides of the intestine in the sixth segment. They are chambered, and the spermatic elements in the hinder chambers are more advanced than those in the anterior chambers. The length of each testis is that of the segment in which they lie.

The vas deferens of each side is a direct continuation of the wall of the testis, and is a very narrow tube running backwards, quite distinct from its fellow of the opposite side, but the two are closely bound together in their hinder parts, and they open behind into the common pouch.

The prostatic glands are a pair of elongated glandular tubes, apparently simple, but seen in sections to be double, though the cavities communicate behind before opening into the common pouch.

The common pouch is a dilatation of the ejaculatory duct at the base of the copulatory organ, and the latter is perhaps derived from a pair of abdominal appendages.

The hinder parts of the vasa deferentia appear to be developed as a forward outgrowth of the ventral wall of the common pouch, and the prostatic glands are lateral outgrowths of the same. The hinder part of each vas deferens is in some Culicidæ expanded to form a vesicula seminalis of considerable size, but this is not the case in Culex.

II. The *female* generative organs are a pair of ovaries, oviducts uniting behind to form a median oviduct, a median copulatory pouch and three spermathecæ opening into the last.

The ovaries correspond in size and position with the testes.

The median oviduct is formed by invagination in the region which I take to be the ninth sternum, while the anus opens at the posterior end of what I take to be the eleventh segment, so that there is no common cloaca. This invagination is already far advanced at the beginning of pupal life (Fig. 7), and during this period it grows forwards, keeping pace with the forward shifting of the last pair of ganglia, and at all stages lying just behind it, till the final ecdysis, when the rapid shifting of the ganglia leaves it behind. Its anterior end is, in the adult, near the anterior end of the seventh segment.

In the youngest pupæ three flattened invaginations, the future spermathecæ, lie upon the dorsal wall of this median oviduct. During the pupal period the anterior end of each becomes spherical and acquires a strong chitinous lining. The anterior ends of these organs remain stationary in the eighth segment throughout.

The bursa copulatrix is a dorsal outgrowth of the invagination which gives rise to the median oviduct, and is a small pouch lying just behind and above the genital aperture.

I am painfully conscious of the fact that the foregoing account of this interesting pupa is far from complete, but the pressure of other work prevents my adding anything considerable to it at present. As soon as I have time to do so, I intend to work out the details of the development of the eye, but fear it will not be possible before next summer.

In conclusion, I would express my best thanks, *firstly*, to the Council of the Owens College, through whose generosity I have been able to leave my work in Manchester; and, *secondly*, to my honoured teacher, Herr Geheimrath Professor Dr. Leuckart, to whom I am indebted for many kind hints, especially as to the literature of the subject, and also for the loan of very numerous books and papers.

I subjoin a list of the books and papers to which I have referred in the foregoing dissertation:—

1. SWAMMERDAM.—"Bibel der Natur."
2. RASCHKE.—"Die larve von Culex nemorosus." Berlin, 1887.
3. A. M. MAYER.—"Researches in Acoustics," "American Journal of Science," 1874.
4. LANDOIS.—"Die Ton- und Stimm-Apparate der Insecten," "Zeitsch. f. wiss. Zool.," xvii., 1867.
5. DIMMOCK.—"Mouth-parts of some Diptera." Boston, 1881.
6. PALMÉN.—"Zur Morphologie des Tracheensystems." Helsingfors, 1877.
7. HUXLEY.—"Anatomy of Invertebrated Animals."
8. MIALL and DENNY.—"The Life-history and Structure of the Cockroach." London, 1886.
9. CHUN.—"Bau, etc., der Rectal-drüsen bei den Insekten." Frankfurt a/M., 1875.
10. GRABER.—"Ueber den propulsatorischen Apparat der Insekten," "Arch. f. Mikr. Anat.," ix., 1873.
11. PATTEN.—"The Eyes of Molluscs and Arthropods," "Mitth. aus d. zool. Stat. zu Neapel," 1886.
12. KOWALEVSKY.—"Ein Beitrag zur Kenntnis der Excretions-Organe," "Biolog. Central-blatt," ix., 1889.
13. JOHNSTON.—"Auditory Apparatus of the Culex Mosquito," "Journ. Microscopical Science" (old series), vol. iii., 1855.

VITA.

I WAS born in Littleborough, in Lancashire, September 6th, 1855. After elementary education in private schools, I spent three and a half years at the Manchester Grammar School; three years as apprentice to a firm of manufacturing pharmaceutical chemists; one year as assistant to an analytical chemist; one year as student of chemistry at the Royal School of Science, South Kensington; one year as science-teacher in a boarding school; three semesters as student of biology and geology at the Royal School of Science, South Kensington, under Professors Huxley, Thistleton-Dyer, McNab, and Judd; and one year at Owens College as student of zoology and embryology, under Professor Milnes Marshall. I then entered the University of Leipzig (1882), and while there was appointed Assistant Lecturer and Demonstrator in Zoology in the Owens College, Manchester, which post I still hold, along with the position of Lecturer of the Victoria University.

The Council of the College generously gave me leave of absence for the whole of the summer session of the present year to enable me to return to Leipzig, where I have made nearly the whole of the observations recorded in the accompanying dissertation.

CHARLES HERBERT HURST.

October 20th, 1889.

DESCRIPTION OF PLATE,

Illustrating Mr. Hurst's Paper on the Pupa of Culex.

Fig. 1. Side view of the male pupa (× 10).

Fig. 2. Ventral view of the female pupa partially extended (× 10).

Fig. 3 to 6. Successive stages in the metamorphosis of the epithelium of the hinder part of the stomach (× 225).

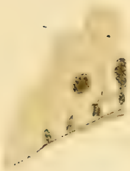
Fig. 7. Sagittal section of a very young female pupa (× 50).

Ant. Antenna. *Ao.* Aorta. *At.* Respiratory siphon. *B.* Buccal chamber. *CG.* Cerebral ganglion. *D.* Gastric pouch. *F.* Fin. *Fe* 1. Femur of the first leg. *G.* Ganglia. *Gn.* Outgrowth of "ninth segment," within which the gonapophyses develop. *Hr.* Halter. *H.* Head. *Ht.* Heart. *In.* Intestine. *Lb.* Labium. *Lbr.*, *Lr.* Labrum. *M.* Malpighian tube. *M.Ap.* Its opening into the intestine. *MS.* Mesosternum. *Mt.* Metasternum. *Mx.* Maxilla (first). *Mxp.* Its palp. *NC.* Nerve commissures and ventral cord. *Oc.* Ocellus. *Od.* Median oviduct. *Op.* Compound eye. *P.* Prosternum. *R.* Rectum. *S.* Aperture of salivary duct. *SD.* Salivary duct. *SG.* Subœsophageal ganglion. *Si.* Larval respiratory siphon introverted into eighth segment. *Sp.* Spermatheca. *St.* Stomach. *Ta* 1, *Ta* 2. Proximal joints of tarsi. *Ti* 1, *Ti* 2, *Ti* 3. Tibiæ. *Tr.* Trachea. *W.* Wing.

I., II., III., etc. First to eighth segments of abdomen.

Fig. 1.

Fig



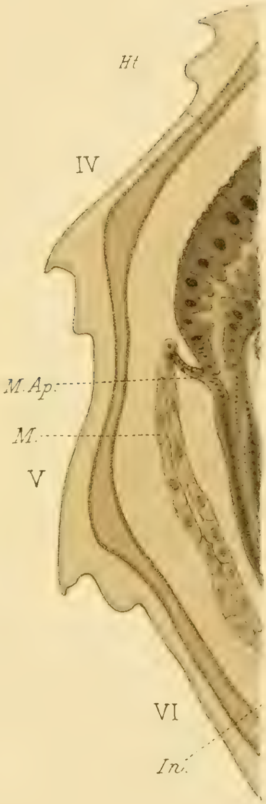


Fig. 2.

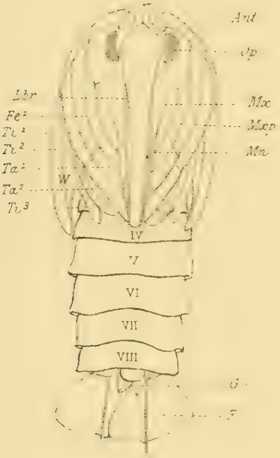
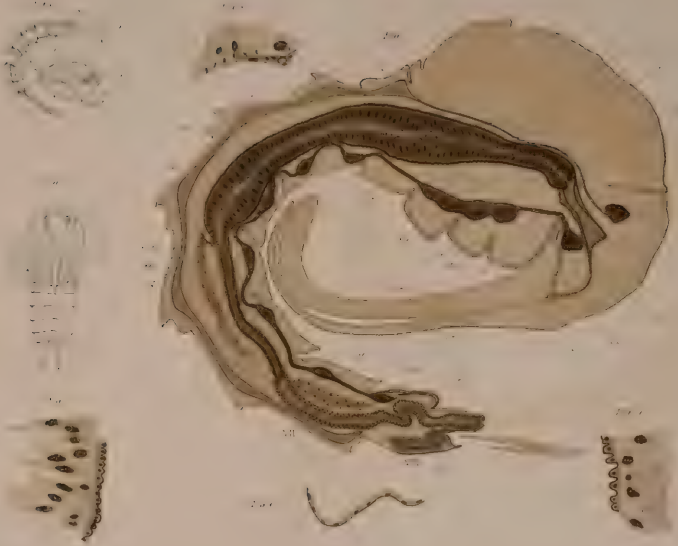


Fig. 5

C. Herbert Hurst del.



C U L E X

Hurst, Charles Herbert, 1856-1898.
The pupal stage of Culex ...